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Signed William Morell

Dated 3 April 2009

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The Patent Office

Cardiff Road
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1. Your reference — JWJ01023GB

2. Patent application number *(The Patent Office will fill in this part)* — 0308852.3 — 16 APR 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

LingVitae AS
Trimveien 6, A562
0372 Oslo
Norway

Patents ADP number *(if you know it)* — 8612582001

If the applicant is a corporate body, give the country/state of its incorporation — Norway

4. Title of the invention — Method

5. Name of your agent *(if you have one)*

Gill Jennings & Every

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number *(if you know it)* — 745002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77

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Continuation sheets of this form	
Description	13
Claim *(s)*	2
Abstract	
Drawing *(s)*	4 +4

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	/
Any other documents *(please specify)*	NO

11. For the applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 16 April 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

JAPPY, John William Graham
020 7377 1377

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Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

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METHOD

Field of the Invention

This invention relates to methods for determining the individual components of a polymer, in particular, determining the sequence of a polynucleotide.

Background to the Invention

Advances in the study of molecules have been led, in part, by improvement in technologies used to characterise the molecules or their biological reactions. In particular, the study of the nucleic acids DNA and RNA has benefited from developing technologies used for sequence analysis and the study of hybridisation events.

The principal method in general use for large-scale DNA sequencing is the chain termination method. This method was first developed by Sanger and Coulson (Sanger *et al.*, Proc. Natl. Acad. Sci. USA, 1977; 74: 5463-5467), and relies on the use of dideoxy derivatives of the four nucleotides which are incorporated into the nascent polynucleotide chain in a polymerase reaction. Upon incorporation, the dideoxy derivatives terminate the polymerase reaction and the products are then separated by gel electrophoresis and analysed to reveal the position at which the particular dideoxy derivative was incorporated into the chain.

Although this method is widely used and produces reliable results, it is recognised that it is slow, labour-intensive and expensive.

US-A-5302509 discloses a method to sequence a polynucleotide immobilised on a solid support. The method relies on the incorporation of 3-blocked bases A, G, C and T having a different fluorescent label to the immobilised polynucleotide, in the presence of DNA polymerase. The polymerase incorporates a base complementary to the target polynucleotide, but is prevented from further addition by the 3'-blocking group. The label of the incorporated base can then be determined and the blocking group removed by chemical cleavage to allow further polymerisation to occur. However, the need to remove the blocking groups in this manner is time-consuming and must be performed with high efficiency.

WO-A-00/39333 describes a method for sequencing polynucleotide by converting the sequence of a target polynucleotide into a second polynucleotide having a defined sequence and positional information contained therein. The sequence information of the target is said to be "magnified" in the second polynucleotide, allowing greater ease of distinguishing between the individual bases on the target molecule. This is achieved using "magnifying tags" which are predetermined nucleic acid sequences. Each of the bases adenine, cytosine, guanine and thymine on the target molecule is represented by an individual magnifying tag, converting the original target sequence into a magnified sequence. Conventional techniques may then be used to determine the order of the magnifying tags, and thereby determining the specific sequence on the target polynucleotide.

In a preferred sequencing method, each magnifying tag comprises a label, e.g. a fluorescent label, which may then be identified and used to characterise the magnifying tag.

Although the method disclosed in this patent publication has many advantages, there is still a need for improved methods for sequencing target polynucleotides.

Summary of the Invention

The present invention is based on the realisation that a target polynucleotide can be converted into a defined sequence and that this defined sequence can be characterised by the incorporation of detectable labels.

According to a first aspect of the invention, a method for determining the sequence of a target polynucleotide comprises the steps of:

(i) converting the target polynucleotide sequence into a second polynucleotide of defined sequence, wherein each of the bases A, T(U), G and C on the target polynucleotide is represented by at least one distinct unit comprising at least a 2 base sequence;

(ii) contacting the second polynucleotide with at least two of the nucleotides dATP, dTTP, dGTP and dCTP, under conditions that permit the polymerisation reaction to proceed, wherein at least one nucleotide comprises a specific detectable label;

(iii) removing any non-incorporated nucleotide and detecting any incorporated nucleotide;

(iv) removing any labels; and

(v) repeating steps (ii) to (iv) to thereby identity the sequence of the different units, and thereby the sequence of the target polynucleotide.

The invention permits the rapid identification of each distinct unit on the second polynucleotide to be determined, which in turn allows each distinct base on the target polynucleotide to be characterised.

Description of the Invention

The invention relies on the conversion of a target polynucleotide into a second polynucleotide having distinct, defined, units of nucleic acid sequence, each unit, or unique combination of units, being representative of a particular base on the target polynucleotide.

The term "polynucleotide" is well known in the art and is used to refer to a series of linked nucleic acid molecules, e.g. DNA or RNA. Nucleic acid mimics, e.g. PNA, LNA (locked nucleic acid) and 2'-O-methRNA are also within the scope of the invention.

The reference to the bases A, T(U), G and C, relate to the nucleotide bases adenine, thymine (uracil), guanine and cytosine, as will be appreciated in the art. Uracil replaces thymine when the polynucleotide is RNA, or it can be introduced into DNA using dUTP, again as well understood in the art.

The second polynucleotide is said to comprise distinct "units" of nucleic acid sequence. Each of the bases A, T(U), G and C on the target is represented by a distinct and predefined unit, or unique combination of units. Each unit will preferably comprise two or more nucleotide bases, preferably from 2 to 50 bases, more preferably 2 to 20 bases and most preferably 4 to 10 bases, e.g. 6 bases. There are at least two different bases contained in each unit. In a preferred embodiment there are three different bases in each unit. The design of the units is such that it will be possible to distinguish the different units during a "read-out" step, involving either the incorporation of detectably labelled nucleotides in a polymerisation reaction, or on hybridisation of complementary

oligonucleotides. For example, each base on the target is represented by a series of bases in a unit, where one base will be complementary to a labelled nucleotide introduced during the read-out step, one base will act as a "spacer" to provide separation between incorporated labels, and one base will act as a stop signal.

In a preferred embodiment, two units of distinct sequence are used to represent all of the four bases on the target. According to this embodiment, the two units can be used as a binary system, with one unit representing "0" and the other representing "1". Each base on the target is characterised by a combination of the two units. For example, adenine may be represented by "0" + "0", cytosine by "0" + "1", guanine by "1" + "0" and thymine by "1" + "1", as shown in Figure 1. It is necessary to distinguish between the units, and so a "stop" signal can be incorporated into each unit. It is also preferable to use different units representing "1" and "0", depending on whether the base on the target (template) polynucleotide is in an odd or even numbered position.

This is demonstrated as follows:

Odd numbered template sequence:
"0" : ATTTTTAT(CC)
"1" : GTTTTTGT(CC)

Even numbered template sequence:
"0" : ACCCCCAC(TT)
"1" : GCCCCCGC(TT)

In this example, the underlined bases are the targets for labelled nucleotides in a polymerase reaction, the bases in parentheses are used as a stop signal, and the remaining bases are to provide separation between the labels.

In odd numbered positions (1, 3, 5, etc) the nucleotide mix, introduced during the polymerase reaction, consists of Fluor X-dUTP, Fluor Y-dCTP and dATP (dGTP is missing from the mix). The complementary base for Fluor Y is

missing for "0", and the complementary base for Fluor X is missing for "1". Accordingly, during a polymerase reaction, if the unit "0" is present, it will be possible to detect this by monitoring for Fluor X, and if "1" is present, by monitoring for Fluor Y.

In all even numbered positions (2, 4, 6, etc) the nucleotide mix consists of the same two fluor-labelled nucleotides, but dGTP is used, not dATP, and one or more T bases define the stop signal.

After each unit has been "read" it is possible to restart the process by introducing the missing complementary nucleotide (eg. either dGTP or dATP) to allow incorporation at the stop sequence. Non-incorporated nucleotides are washed away prior to the next read-out step.

The reaction can be extended to a 4-colour system using four different signals (eg. fluors). The four signals are obtained by using two different sets of two labelled nucleotides (eg. dUTP and dCTP). In order to provide the proper spacing between bulky signal groups, a two-step fill-in reaction is required: The first reaction fills in signal 1 and 2 for Adenine and Cytosine, respectively (dGTP is lacking). After a wash step to remove all nucleotides, a second fill-in reaction is performed to fill in signals 3 and 4 for Thymine and Guanine, respectively (this time dATP is lacking).

"A": ATTTTTATCCCCCCCCCTT (Signal 1, eg., Em 159)
"C": GTTTTTGTCCCCCCCCCTT (Signal 2, eg., Em 550)
"T": TTTTTTTTCCACCCCCATT (Signal 3, eg., Em 600)
"G": TTTTTTTTCCGCCCCCGTT (Signal 4, eg., Em 650)

In the design of the units, it is preferable to have a spacing of between 6-12 bases between the incorporated labels.

The target polynucleotide may be converted into the second polynucleotide using methods known in the art. For example, the conversion method disclosed in WO-A-00/39333, using restriction enzymes, may be adopted. For example, the target polynucleotide may be ligated into a vector which carries a class IIS restriction site close to the point of insertion, or the target polynucleotide may be engineered to contain such a site. The appropriate

class IIS restriction enzyme is then used to cleave the restriction site, resulting in an overhang in the target sequence.

Appropriate adapters which contain one or more of the units may then be used to bind to one or more of the bases of the overhang. Once the overhang of the adapter and the cleaved vector have been hybridised, these molecules may be ligated. This will only be achieved where full complementarity along the full extent of the overhang is achieved. Blunt end ligation may then be effected to join the other end of the adapter to the vector. By appropriate placement of a further class II restriction site (or other appropriate restriction enzyme site), which may be same or different to the previously used enzyme, cleavage may be effected such that an overhang is created in the target sequence downstream of the sequence to which the first adapter was directed. In this way, adjacent or overlapping sequences may be consecutively converted into sequences carrying the units of defined sequence.

Using this conversion system, the second polynucleotide is formed comprising a binary system of defined units, wherein two consecutive units are used to define a particular base on the target sequence.

Having converted the target sequence into the sequential units of the second polynucleotide, the sequence of the units may then be determined, to thereby determine the target polynucleotide sequence.

This may be achieved as discussed above using the polymerase reaction to incorporate bases complementary to those on the second polynucleotide, using either selected, detectably-labelled nucleotides or nucleotides that incorporate a group for subsequent indirect labelling, and monitoring any incorporation event.

The polymerase reaction is carried out under conditions that permit the controlled incorporation of complementary nucleotides one unit at a time. This enables each unit to be categorised by the detection of an incorporated label. As each unit will include a "stop" sequence, it is possible to control incorporation by supplying only those nucleotides required for incorporation onto the first unit, as described above. As each unit is recognised by a specific label, it is possible to distinguish between two different units (0 and 1) within each cycle. This

enables detection of any incorporated label, and allows the identification and position of the unit to be determined.

The method may be carried out as follows:

(i) contacting the second polynucleotide with at least two of the nucleotides dATP, dTTP, dGTP and dCTP, under conditions that permit the polymerisation reaction to proceed, wherein at least one nucleotide comprises a detectable label specific for that nucleotide;

(ii) removing any non-incorporated nucleotides and detecting any incorporation events;

(iii) removing the labels from incorporated nucleotide; and

(iv) repeating steps ii) to iv), to thereby identify the different units, and thereby the sequence of the target polynucleotide.

The choice of polymerase and detectable label will be apparent to the skilled person. The following is used as a guide only:

a) Klenow and Klenow (exo-) can efficiently incorporate Tetramethylrhodamine-4-dUTP and Rhodamin-110-dCTP (Amersham Pharmacia Biotech) (Brakmann and Nieckchen, 2001, Brakmann and Löbermann, 2000).

b) Vent, Taq and Tgo DNA polymerase can efficiently incorporate dioxigenin and fluorophores like AMCA, Tetramethylrhodamin, fluorescein and Cy5 without spacing at least up to a few positions (Augustin *et al*, 2001).

c) T4 DNA polymerase is efficient in filling-in fluorophore labelled nucleotides.

The preferred polymerases are Klenow Large fragment (exo-) and T4 DNA polymerase.

In a preferred embodiment, multiple second polynucleotides are immobilised on a support material. This places each polynucleotide in a fixed position, and allows the sequence of each polynucleotide to be determined by aligning consecutive images of the support material to establish the order in which the labels were detected.

Polynucleotides may be attached to support materials by recognised means, including the use of biotin-avidin interactions. Methods for immobilising

polynucleotides on support materials are well known in the art, and include photolithographic techniques and techniques that rely on "spotting" individual polynucleotides in defined positions on a support material. Immobilisation may also be carried out by the random distribution of polynucleotides on microbeads, nanoparticles and planar surfaces.

Immobilisation may be by specific covalent or non-covalent interactions. The interaction should be sufficient to maintain the polynucleotides on the support during washing steps to remove unwanted reaction components. Immobilisation will preferably be at either the 5' or 3' position, so that the polynucleotide is attached to the support at the end only. However, the polynucleotide may be attached to the support at any position along its length, the attachment acting to tether the polynucleotide to the support.

The skilled person will appreciate the appropriate means to immobilise the polynucleotide to the support material. Suitable coatings may be applied to the support to facilitate immobilisation, as will be appreciated by the skilled person. Suitable coatings include epoxy coatings (eg. 3-glycidyloxypropyltrimethoxysilane), superaldehyde coating, mercaptosilane, and isothiocyanate. Alternatively, several linker groups may be used, including PAMAM dendritic structures (Benters *et al.*, Chem Biochem., 2001; 2: 686-694) and the immobilisation linkers described in Zhao *et al.*, Nucleic Acids Research, 2001; 29(4): 955-959.

Suitable support materials are known in the art, and include glass slides, ceramic and silicon surfaces and plastics materials. The support is usually a flat (planar) surface.

The second polynucleotide may be immobilised on the support material to form polynucleotide arrays which may form a random or ordered pattern on the solid support. Preferably, the arrays that are used are single molecule arrays that comprise polynucleotides in distinct optically resolvable areas, e.g. as disclosed in WO-A-00/06770.

To carry out the polymerase reaction it will usually be necessary to first anneal a primer sequence to the polynucleotide, the primer sequence being recognised by the polymerase enzyme and acting as an initiation site for the

subsequent extension of the complementary strand. The primer sequence may be added as a separate component with respect to the polynucleotide, which comprises a complementary sequence that allows the primer to anneal.

Other conditions necessary for carrying out the polymerase reaction, including temperature, pH, buffer compositions etc., will be apparent to those skilled in the art. The polymerisation step is likely to proceed for a time sufficient to allow incorporation of bases to the first unit. Non-incorporated nucleotides are then removed, for example, by subjecting the array to a washing step, and detection of the incorporated labels may then be carried out.

In a preferred embodiment, the label is a fluorescent moiety. Many examples of fluorophores that may be used are known in the prior art, and include:

Alexa dyes (Molecular Probes)

BODIPY dyes (Molecular Probes)

Cyanine dyes (Amersham Biosciences Ltd.)

Tetramethylrhodamine (Perkin Elmer, Molecular Probes, Roche Diagnostics)

Coumarin (Perkin Elmer)

Texas Red (Molecular Probes)

Fluorescein (Perkin Elmer, Molecular Probes, Roche Diagnostics)

Phycobiliproteins (Molecular Probes)

Nanoparticles

The attachment of a suitable fluorophore to a nucleotide can be carried out by conventional means. Suitably labelled nucleotides are also available from commercial sources. The label is attached in a way that permits removal, after the detection step. This may be carried out by any conventional method, including:

I. Attacking the signal itself:

a) Bleaching

  i) Photobleaching

  ii) Chemical bleaching

a) Quenching of fluorescence

i) By antibodies raised against the fluor (eg. anti-fluorescein, anti-Oregon green)

ii) By FRET (the incorporation of a quencher next to a signal can be used to quench the signal, eg. Taqman strategy)

c) Cleavage of signal

i) Chemical cleavage (eg. reduction of a disulfide bridge between the base and the signal)

ii) Photocleavage (eg. introduction of a nitrobenzyl or tert-butylketon group)

iii) Enzymatic (eg. α-chymotryspin digestion of peptide linker)

II. The signal bearing nucleotide:

c) Exonucleolytic removal

i) 3'-5' Exonucleolytic degradation of filled-in nucleotides (eg. exonuclease III or by activating the 3'-5' exonucleolytic activity of DNA polymerase when there is an absence of certain nucleotides)

d) Restriction enzyme digestion

i) Digestion of double-stranded DNA bearing the signal (eg. ApaI, DraI, SmaI sites which can be incorporated at the stop signals).

The preferred method is by photo or chemical cleavage.

When the label is a fluorophore, the fluorescent signal generated on incorporation may be measured by optical means, e.g. by a confocal microscope. Alternatively, a sensitive 2-D detector, such as a charge-coupled detector (CCD), can be used to visualise the individual signals generated, as shown in Figure 2.

The general set-up for optical detection is as follows:

Microscope:	Epi-fluorescence
Objective:	Oil emersion (100X, 1.3 NA)
Light source:	Lasers or lamp
Filters:	Bandpass
Mirrors:	Dichroic mirror and dichroic wedge
Detectors:	Photomultiplier tubes (PMT) or CCD camera

Variants may also be used, including:

A. Confocal Laser Scanning Microscopy (CLSM)

Light source: One or more lasers

Background reduction: One or several pinhole apertures

Detection: a) A single pinhole: Photomultiplier tube (PMT) detectors for different fluorescent wavelengths [The final image is built up point by point and over time by a computer].

b) Several thousands pinholes (spinning Nipkow disk): CCD camera detection of image [The final image can be directly recorded by the camera]

B. Two-Photon (TPLSM) and Multiphoton Laser Scanning Microscopy

Light source: One or more lasers

Background control: No pinhole required

Detection: CCD camera (video and digital imaging systems)

C. Total Internal Reflection Fluorescence Microscopy (TIRFM)

Light source: One or more lasers

Background control: No pinhole required

Detection: CCD camera (video and digital imaging systems)

The preferred methods are TIRFM and confocal microscopy.

The following Examples illustrate the invention.

Example 1 Primer extension:

A target polynucleotide is converted into a series of second polynucleotides using the methods disclosed in WO-A-00/39333. Four defined second polynucleotides are used to represent 0 and 1 units in both even and odd numbered positions. The 0- and 1- units have the sequence ATTTTTATCC and GTTTTTGTCC, respectively, in odd numbered positions, while their codings are ACCCCCACTT and GCCCCCGCTT, respectively, in even numbered positions.

5'-amino labeled single stranded second polynucleotides are generated from double-stranded template (end product of the conversion) by asymmetric PCR using 5'-amino labeled primer, DNA polymerase and dNTPs. A common primer is annealed to the amino-labeled second polynucleotides and the molecule is immobilized to an expoxy-coated glass slide via the amino-group.

Conditions are chosen to avoid aggregation of the molecules (e.g. low salt) and to ensure single molecule resolution by fluorescence microscopy.

A buffer solution "odd" containing Alexa-488-dUTP (or Cy3-dUTP), Alexa-647-dCTP (or Cy5-dCTP), dATP (dGTP missing) and DNA polymerase (Klenow or T4 DNA polymerase) is added to the slides. The fluorophore labeled nucleotides contain a photocleavable linker inserted between the fluorochrome and the base. The slides are incubated for a few minutes for the polymerase reaction to occur. After a washing procedure to remove DNA polymerase and unincorporated nucleotides, a series of images covering the entire slide are captured using TIR fluorescence microscopy and ICCD-camera detection. The label is removed by photocleavage (340 nm for 2-nitrobenzyl linker), and the slide is ready for a second round after a brief wash to remove the cleaved label. A buffer solution "even" containing exactly the same constituents as used in "odd" only with dGTP replacing dATP, is added to the slide to start the fill-in of position two. Detection and removal of signal proceeds as described for cycle one. By cycling between these two buffer systems, the units are determined in a controlled manner.

Example 2 Oligonucleotide Hybridisation:

The same target polynucleotide is sequenced using a method based on hybridisation. 0- and 1- units are built up from 15-20 bp sequences that define both the base and its position. Thus, a second polynucleotide containing 40 units (i.e. 20 bp from the target polynucleotide) is built up from a repertoir of 2 x 40 different 15-20 bp sequences having similar melting characteristics.

5'-amino labeled single stranded second polynucleotides are generated from double-stranded template (end product of the conversion) by asymmetric PCR using 5'-amino labeled primer, DNA polymerase and dNTPs. The second polynucleotides are immobilized to a glass slide via the amino-group, using a glass coating that can withstand several cycles of hybridization and denaturation (PAMAM dendrimer coated glass slide). Conditions are chosen to avoid aggregation of the molecules (e.g. low salt) and to ensure single molecule resolution by fluorescence microscopy.

Two different fluorophore-labeled oligonucleotides representing 0 and 1,

respectively in position one are hybridised to the immobilised polynucleotides using stringent conditions (to avoid mis-hybridisation). After several stringent washes to remove unhybridised oligos, images are captured as described in Example 1.

5

CLAIMS

1. A method for determining the sequence of a target polynucleotide comprising the steps of:

(i) converting the target polynucleotide sequence into a second polynucleotide of defined sequence, wherein each of the bases A, T(U), G or C on the target polynucleotide is represented by at least one distinct unit comprising at least a two base sequence;

(ii) contacting the second polynucleotide with at least two of the nucleotides dATP, dTTP (dUTP), dGTP and dCTP, under conditions that permit the polymerisation reaction to proceed, wherein at least one nucleotide comprises a detectable label specific for that nucleotide;

(iii) removing any non-incorporated nucleotides and detecting any incorporation events;

(iv) removing any labels; and

(v) repeating steps (ii) to (iv) to thereby identity the different units, and thereby the sequence of the target polynucleotide.

2. A method according to claim 1, wherein step (ii) comprises three nucleotides, two of which are differently labelled.

3. A method according to claim 1 or claim 2, wherein the label is a fluorophore.

4. A method according to claim 3, wherein the fluorophore is Alexa-red or Alexa-green.

5. A method according to any preceding claim, wherein each distinct unit is separated by one or more bases not present in the preceding unit.

6. A method according to any preceding claim, wherein the second polynucleotide is immobilised on a support material.

7. A method according to claim 6, wherein the immobilised polynucleotide form an array on the support material, the array having a density that permits individual resolution of a detectable label on each polynucleotide.

8. A method according to any preceding claim, wherein detection is carried out by optical microscopy.

9. A method according to any preceding claim, wherein each of the bases A, T(U), G and C on the target polynucleotide is represented by a combination of two sequential units on the second polynucleotide, with each base represented by a different combination of the two units.

10. A method for determining the sequence of a target polynucleotide comprising the steps of:

i) converting the target polynucleotide sequence into a second polynucleotide of defined sequence, wherein each of the bases A, T(U), G or C on the target polynucleotide is represented by at least one distinct unit comprising at least a two base sequence;

ii) contacting the second polynucleotide with an oligonucleotide under hybridising conditions, the oligonucleotide being complementary to a unit on the second polynucleotide and being detectably labelled;

iii) removing any non-hybridised oligonucleotides and detecting an hybridisation event;

iv) removing any label(s); and

v) repeating steps (ii) to (iv) to thereby identify the different units, and thereby the sequence of the target polynucleotide.

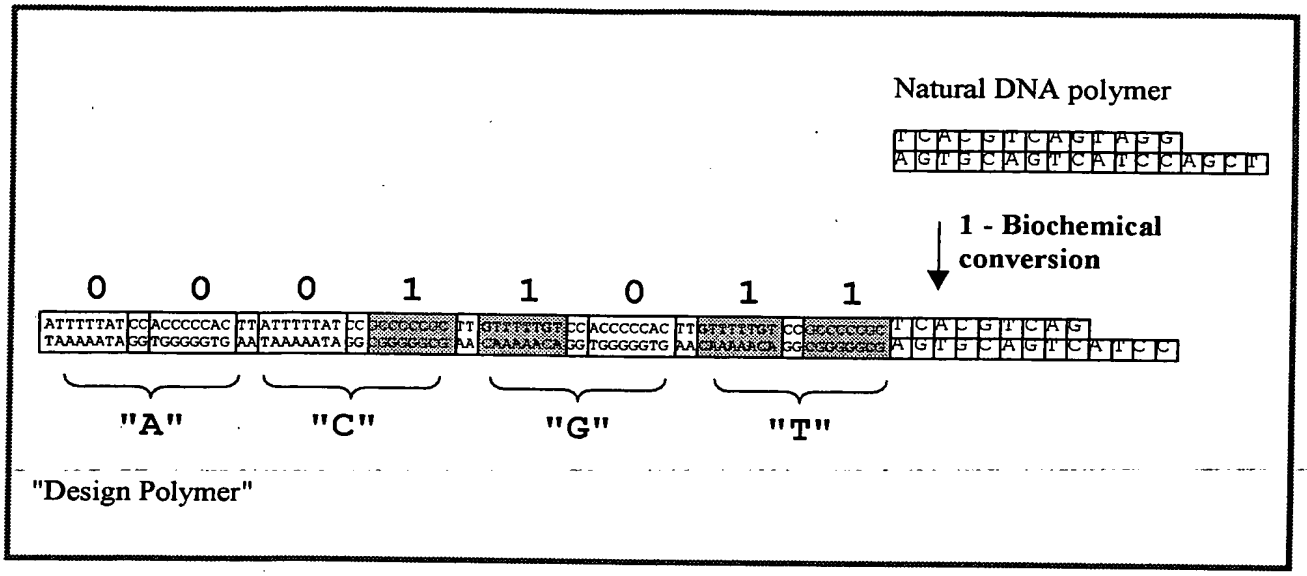
Natural DNA polymer
1 - Biochemical conversion
0 0 0 1 1 0 1 1
"A"
"C"
"G"
"T"
"Design Polymer"

Figure 1

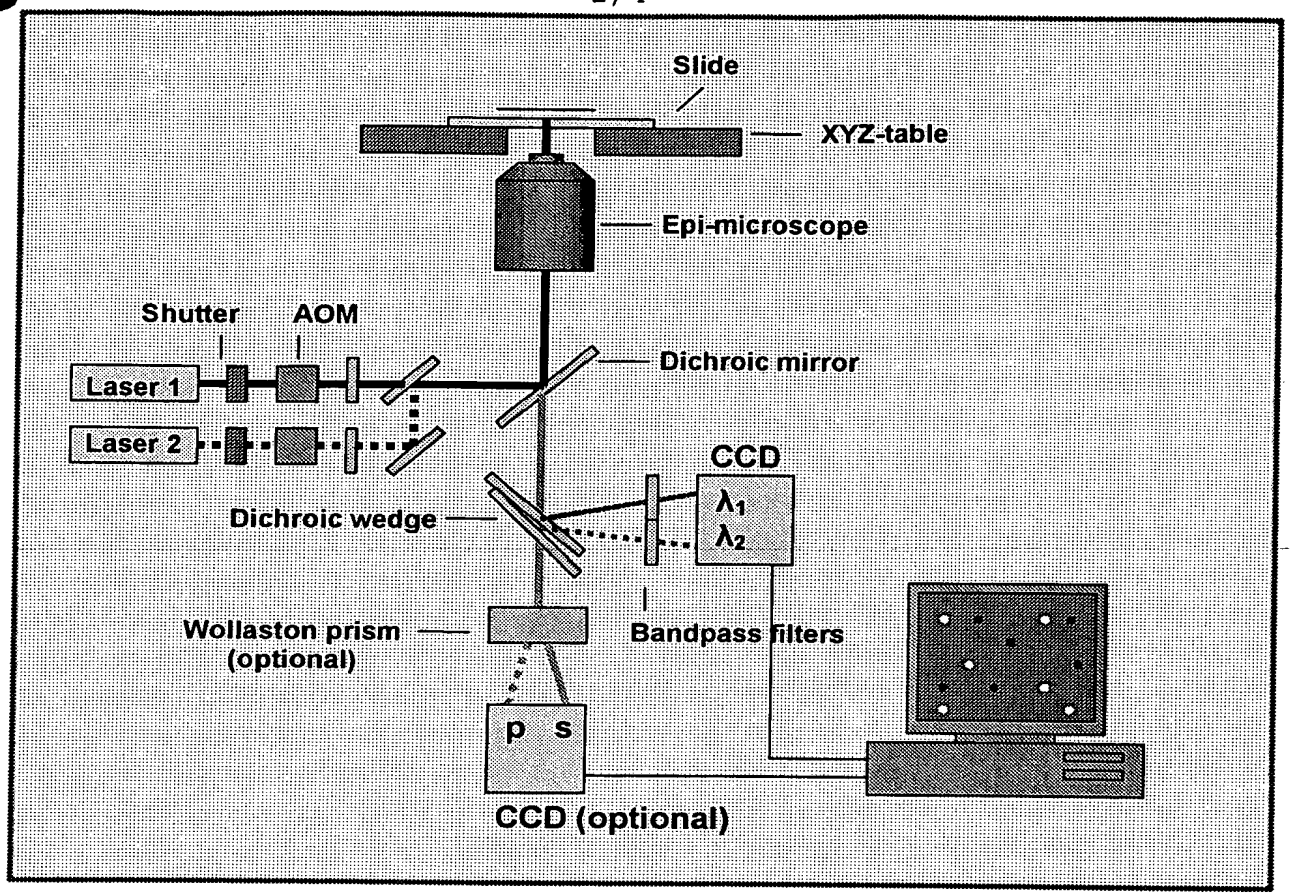
Slide
XYZ-table
Epi-microscope
Shutter
AOM
Laser 1
Laser 2
Dichroic mirror
CCD
λ1
λ2
Dichroic wedge
Wollaston prism
(optional)
Bandpass filters
p
s
CCD (optional)

Figure 2

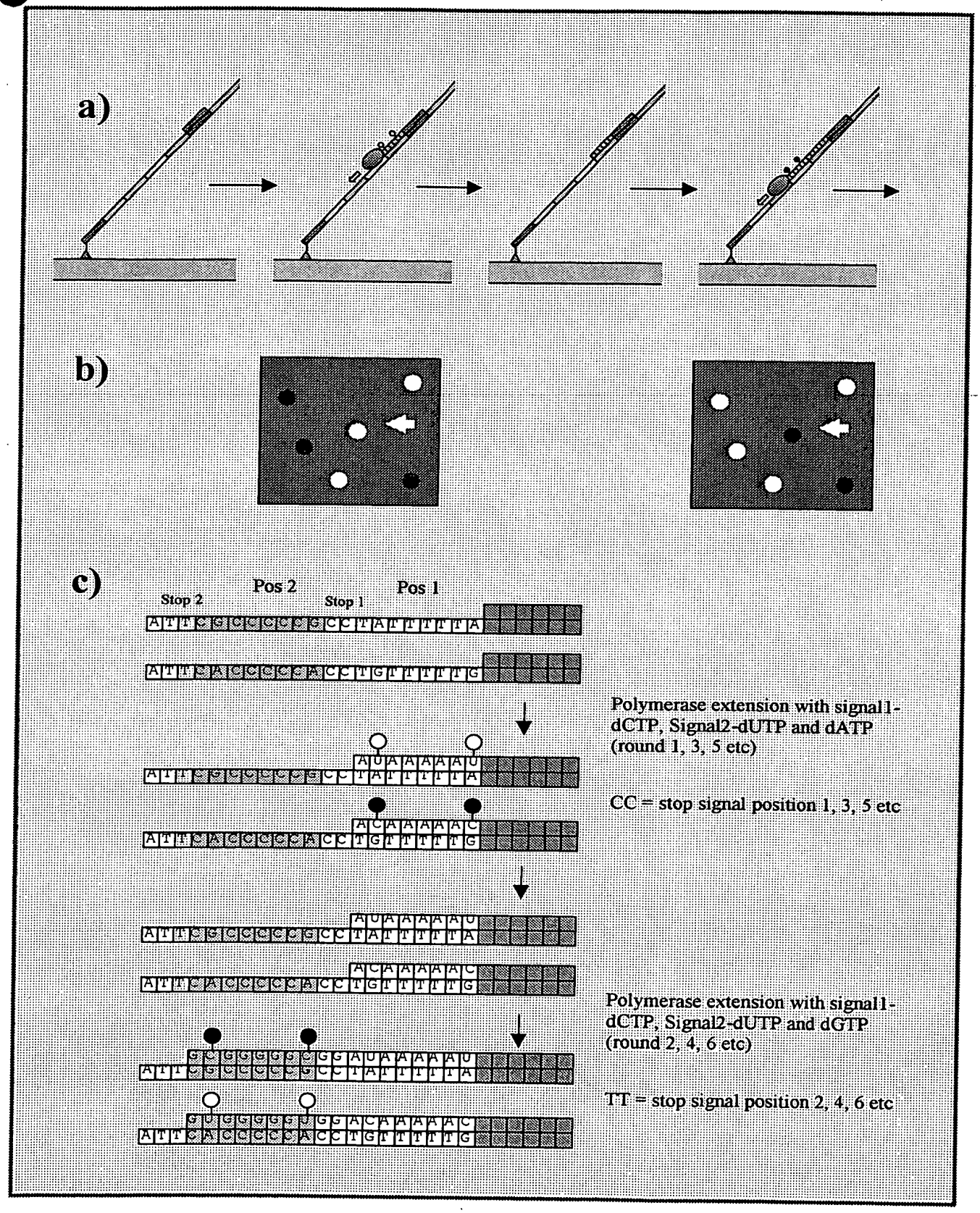
a)
b)
c)
Stop 2
Pos 2
Stop 1
Pos 1
Polymerase extension with signal1-dCTP, Signal2-dUTP and dATP (round 1, 3, 5 etc)
CC = stop signal position 1, 3, 5 etc
Polymerase extension with signal1-dCTP, Signal2-dUTP and dGTP (round 2, 4, 6 etc)
TT = stop signal position 2, 4, 6 etc

Figure 3

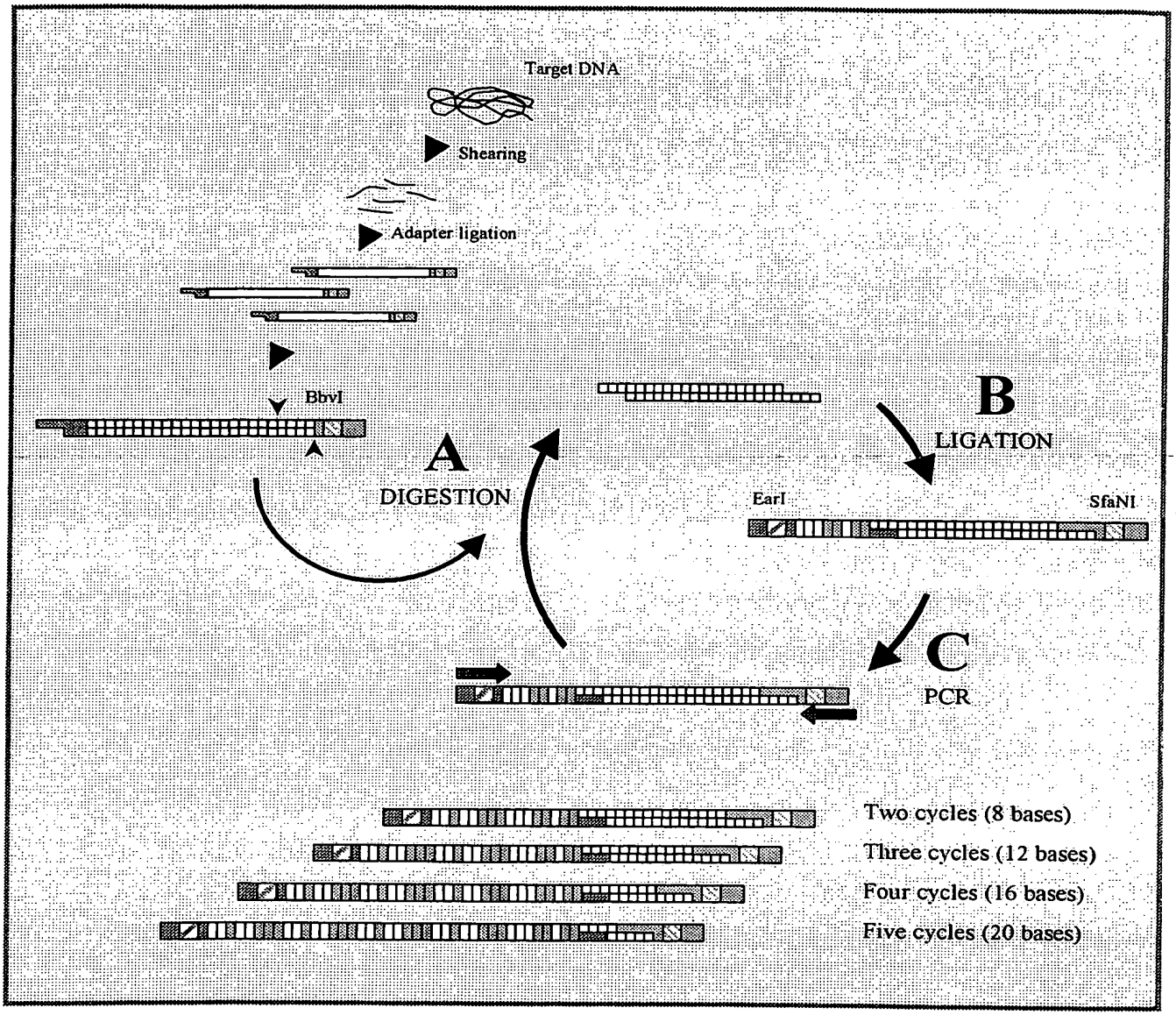
Target DNA
Shearing
Adapter ligation
BbvI
A
DIGESTION
B
LIGATION
EarI
SfaNI
C
PCR
Two cycles (8 bases)
Three cycles (12 bases)
Four cycles (16 bases)
Five cycles (20 bases)

Figure 4

Application No. (if known): 10/553,505 Attorney Docket No.: 30986/41550

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